

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings of claims in the application:

LISTING OF CLAIMS:

1. (currently amended) Method for analyzing adducts in a fluid and/or solid material suspected of containing said adducts, wherein said adduct is an N-adducted amino acid or adducted N-terminal peptide/protein, comprising the following steps:

a) bringing said fluid and/or solid material in direct contact with an isothiocyanate reagent ~~containing a fluorescent and, preferably, also an ionizable moiety, with the exception of a reagent in which the isothiocyanate group is directly bound to an unsubstituted phenyl or pentafluorophenyl group~~ wherein said reagent is an isothiocyanate reagent containing a fluorescent moiety and an ionizable moiety selected from the group consisting of FITC, DNITC and DABITC or a derivative thereof;

b) allowing said reagent to react with adducted N-terminals in proteins or peptides present in said fluid and/or solid material;

c) separating the analytes formed from the reaction mixture; and

d) detecting the analytes formed, and optionally visualizing the result, wherein step c) is performed using LC and step d) is performed using MS detection.

2. (original) A method according to claim 1 wherein the detection step d) is followed by a step e) comparing the results from the detection step d) with previously obtained results, obtained using steps a) - d), which previously obtained

results emanate from a standard material formed from the adduct under scrutiny, and optionally calculating a quotient between said results and optionally presenting said quotient visually.

3. (currently amended) A method according to claim 1 wherein said adducted N-terminals have their adducts attached to a secondary N-terminal ~~valin~~ valine in hemoglobin, a secondary N-terminal asparagine in serum albumin or to a secondary N-terminal glycine in myoglobin, ~~preferably an N-terminal valin.~~

4. (original) A method according to claim 1 wherein said adduct is a globin adduct.

5. (original) A method according to claim 1 wherein said adduct is a hemoglobin or a myoglobin adduct.

6. (original) A method according to claim 1 wherein said adduct is a serum albumin adduct.

7-9. (canceled)

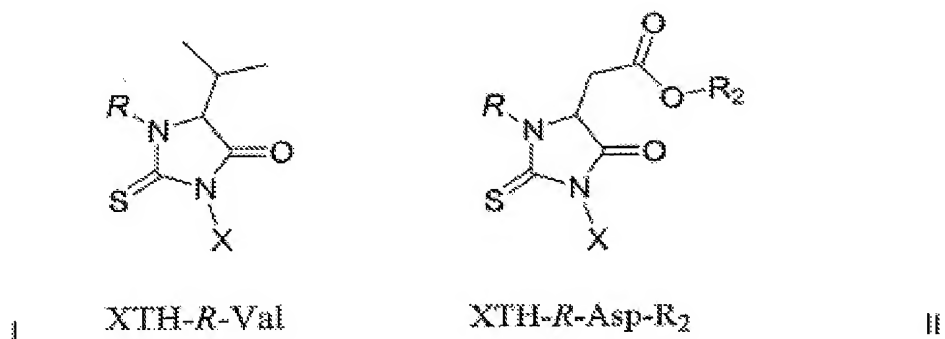
10. (currently amended) A method according to claim 1 wherein said reagent is ~~an isothiocyanate reagent containing a fluorescent moiety and an ionizable moiety, preferably selected from the group consisting of 4 isothiocyanato benzoic acid, 4 isothiocyanato naphthalene 1 carboxylic acid, 10 isothiocyanato anthracene 9 carboxylic acid, (4 isothiocyanato phenyl) dimethylamine, 9 isothiocyanato acridine, 4 isothiocyanato quinoline, malachite green isothiocyanate, FITC, DNITC and DABITC or a derivative thereof; most preferably, FITC, DNITC and DABITC or a derivative thereof; especially most preferably FITC.~~

11-15. (canceled)

16. (previously presented) A method according to claim 11 wherein step c) is preceded by a step for enriching the analyte present.

17. (currently amended) A method according to claim 16 wherein said enrichment step preceding step c) is performed using size-discriminating ultrafiltration, ~~preferably followed by an ion-exchanging step,~~ or ultracentrifugation, ~~preferably followed by an ion-exchanging step.~~

18. (currently amended) A method according to claim 1 wherein said analyte is a compound according to formula I or II, or a derivative thereof:



wherein R represents any adduct (e.g., alkyl and aryl or substituted analogues thereof, with the exception for hydrogen) and X represents a moiety of any isothiocyanate reagent utilized in which the isothiocyanate group is directly bound to an aromatic ring or an aromatic ring system providing fluorescent and/or ionizable properties to the analyte, ~~which~~ with the exception that X is not a phenyl, 4-bromophenyl, 4-methoxyphenyl or pentafluorophenyl group, and R₂ represents hydrogen, an alkyl,

aryl, carboxyl or benzyl group or substituted analogues thereof;
or a carboxyl anion group.

19. (currently amended) A method according to claim 1
wherein detection of the analyte in step d) is performed at a pH
above 5, ~~preferably at a pH of approximately 7.~~

20. (canceled)

21. (currently amended) A method according to claim 1
wherein said fluid and/or solid material is blood or processed
blood, ~~preferably of human origin,~~ which has been obtained at an
earlier stage, ~~preferably contained in a container, most
preferred a tube.~~

22. (canceled)

23. (previously presented) A method according to claim 21
wherein the blood is processed either by centrifugation, washing
and lysating, or lysating only.

24. (currently amended) A method according to claim 23
wherein said centrifugation, washing and lysating is followed by
heating at approximately 70°C, ~~preferably during approximately 1 h.~~

25. (currently amended) A method according to claim 23
wherein said lysating only, is followed by heating at
approximately 38°C, ~~preferably during approximately 18 h.~~

26. (canceled)

27. (currently amended) A method according to claim 24
wherein the heating is followed by step c) as set out in claim 1

wherein the separation is performed by size-discriminating ultra filtration in a size-discriminating ultra filtration tube and whereupon the analyte is being bound to an ion exchange resin in said tube and thereupon purifying said analyte.

28. (original) A method according to claim 27 wherein the purifying of said analyte is performed by first washing the resin to which the analyte is bound and release the analyte from the resin preferably by adding an acid to said resin, and subsequently filter the resin off giving the analyte in the remaining filtrate.

29. (currently amended) A method according to claim 28 wherein the detecting as set out in step d) of claim 1 is performed by using ~~CE-LIF or~~ LC-MS/MS, ~~preferably LC-MS/MS.~~

30. (original) A method according to claim 29 wherein alkalization of the detached analytes is performed before detecting using CE-LIF.

31. (currently amended) A method according to claim 25 wherein the heating is followed by step c) as set out in claim 1 wherein the separation is performed by size-discriminating ultra filtration in a size-discriminating ultra filtration tube and wherein the analyte is free in solution and present in the filtrate.

32. (currently amended) A method according to claim 31 wherein the detecting as set out in step d) of claim 1 is performed by using ~~CE-LIF or~~ LC-MS/MS.

33. (currently amended) A method for manufacturing a standard material for use in a method according to claim 1 ~~consisting of~~ comprising the following steps:

i) reacting an N-substituted amino acid or an adducted N-terminal in a protein or a peptide with a reagent wherein said reagent is an isothiocyanate reagent containing a fluorescent and/or an ionizable moiety selected from the group consisting of FITC, DNITC and DABITC or a derivative thereof, ~~with the exception of a reagent in which the isothiocyanate group is directly bounded to an unsubstituted phenyl or pentafluorophenyl group;~~ and

ii) purifying the analyte, which is ~~preferably~~ a thiohydantoin analyte formed, by, e.g., separating the unreacted compound from the reaction mixture.

34. (currently amended) A method according to claim 33 wherein said adducted N-terminals have their adducts attached to a secondary N-terminal ~~valin~~ valine in hemoglobin, a secondary N-terminal asparagine in serum albumin or a secondary N-terminal glycine in myoglobin, ~~preferably an N-terminal valin.~~

35. (original) A method according to claim 33 wherein said adduct is a globin adduct.

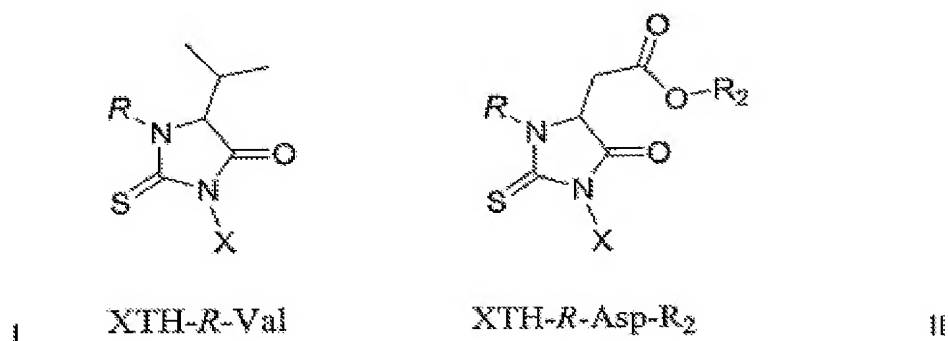
36. (original) A method according to claim 35 wherein said adduct is a hemoglobin or a myoglobin adduct.

37. (original) A method according to claim 33 wherein said adduct is a serum albumin adduct.

38-40. (canceled)

41. (currently amended) A method according to claim 33 wherein said reagent is ~~an isothiocyanate reagent containing a fluorescent moiety and an ionizable moiety, preferably selected from the group consisting of 4 isothiocyanato benzoic acid, 4 isothiocyanato naphthalene 1 carboxylic acid, 10 isothiocyanato anthracene 9 carboxylic acid, (4 isothiocyanato phenyl) dimethylamine, 9 isothiocyanato acridine, 4 isothiocyanato quinoline, malachite green isothiocyanate, FITC, DNITC and DABITC or a derivative thereof; most preferably, FITC, DNITC and DABITC or a derivative thereof; and especially most preferably FITC.~~

42. (currently amended) A method according to claim 33 wherein said analyte is a compound according to formula I or II or a derivative thereof;



wherein R represents any adduct (e.g., alkyl and aryl or substituted analogues thereof, with the exception of hydrogen) and X represents a moiety of any isothiocyanate reagent utilized in ~~which~~ with the isothiocyanate group is directly bound to an aromatic ring or an aromatic ring system, thereby providing fluorescent and/or ionizable properties to the analyte, with the exception that X is not a phenyl, 4- bromophenyl, 4-methoxyphenyl or pentafluorophenyl group, and R₂ represents hydrogen; an alkyl,

aryl, carboxyl or benzyl moiety or substituted analogues thereof;
or a carboxyl anion group.

43. (currently amended) A method according to claim 33 wherein said analyte is a compound selected from the group consisting of ~~3-[4-(4-dimethylamino-phenylazo)-phenyl]-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DABTH-MeVal); 3-(4-dimethylamino-naphthalen-1-yl)-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DNTH-MeVal); fluorescein, 5-(4-isopropyl-3-methyl-2-thioxo-imidazolidin-5-one) (FTH-MeVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-AAVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-2-hydroxy-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-GAVal); fluorescein, 5-[4-isopropyl-3-(2-hydroxyoctadecyl)-2-thioxo-imidazolidin-5-one] (FTH-HOC₁₈Val); fluorescein, 5-[4-isopropyl-3-(2-hydroxy-propyl)-2-thioxo-imidazolidin-5-one] (FTH-HOPrVal); fluorescein, 5-[4-isopropyl-3-[17-(1,5-dimethyl-hexyl)-3,5 and/or 6-dihydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-5 and/or 6-yl]-2-thioxo-imidazolidin-5-one] (FTH-CholEOVal) and fluorescein, 5-[4-isopropyl-3-(2,3,4,5,6-pentahydroxy-hexyl)-2-thioxo-imidazolidin-5-one] (FTH-GIeVal)~~ 3-[4-(4-dimethylamino-phenylazo)-phenyl]-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DABTH-MeVal); 3-(4-dimethylamino-naphthalen-1-yl)-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DNTH-MeVal); fluorescein, 5-(4-isopropyl-3-methyl-2-thioxo-imidazolidin-5-one) (FTH-MeVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-AAVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-2-hydroxy-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-GAVal); fluorescein, 5-[4-isopropyl-3-(2-hydroxyoctadecyl)-2-thioxo-imidazolidin-5-one] (FTH-HOC₁₈Val); fluorescein, 5-[4-isopropyl-3-(2-hydroxy-propyl)-2-thioxo-imidazolidin-5-one] (FTH-HOPrVal); fluorescein, 5-[4-isopropyl-3-[17-(1,5-dimethyl-hexyl)-3,5 and/or

6-dihydroxy-10,13-dimethyl-hexadecahydro-
cyclopenta[a]phenanthren-5 and/or 6-yl))-2-thioxo-imidazolidin-5-
one} (FTH-CholEOVal) and fluorescein, 5-[4-isopropyl-3-
(2,3,4,5,6-pentahydroxy-hexyl)-2-thioxo-imidazolidin-5-one] (FTH-
GlcVal).

44. (previously presented) A standard material obtainable by the method according to claim 33.

45. (currently amended) A compound selected from the group consisting of ~~3-[4-(4-dimethylamino-phenylazo)-phenyl]-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DABTH-MeVal); 3-(4-dimethylamino-naphthalen-1-yl)-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DNTH-MeVal); fluorescein, 5-(4-isopropyl-3-methyl-2-thioxo-imidazolidin-5-one) (FTH-MeVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-AAVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-2-hydroxy-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-GAVal); fluorescein, 5-[4-isopropyl-3-(2-hydroxyoctadecyl)-2-thioxo-imidazolidin-5-one] (FTH-HOCi8Val); fluorescein, 5-[4-isopropyl-3-(2-hydroxy-propyl)-2-thioxo-imidazolidin-5-one] (FTH-HOPrVal); fluorescein, 5-[4-isopropyl-3-[17-(1,5-dimethyl-hexyl)-3,5 and/or 6-dihydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-5 and/or 6-yl))-2-thioxo-imidazolidin-5-one] (FTH-CholEOVal) and fluorescein, 5-[4-isopropyl-3-(2,3,4,5,6-pentahydroxy-hexyl)-2-thioxo-imidazolidin-5-one] (FTH-GlcVal)~~ 3-[4-(4-dimethylamino-phenylazo)-phenyl]-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DABTH-MeVal); 3-(4-dimethylamino-naphthalen-1-yl)-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DNTH-MeVal); fluorescein, 5-(4-isopropyl-3-methyl-2-thioxo-imidazolidin-5-one) (FTH-MeVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-AAVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl

-2-hydroxy-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-GAVal); fluorescein, 5-[4-isopropyl-3-(2-hydroxyoctadecyl)-2-thioxo-imidazolidin-5-one] (FTH-HOC₁₈Val); fluorescein, 5-[4-isopropyl-3-(2-hydroxy-propyl)-2-thioxo-imidazolidin-5-one] (FTH-HOPrVal); fluorescein, 5-{4-isopropyl-3-[17-(1,5-dimethyl-hexyl)-3,5 and/or 6-dihydroxy-10,13-dimethyl-hexadecahydro cyclopenta[a]phenanthren-5 and/or 6-yl])-2-thioxo-imidazolidin-5-one} (FTH-CholEOVal) and fluorescein, 5-[4-isopropyl-3-(2,3,4,5,6-pentahydroxy-hexyl)-2-thioxo-imidazolidin-5-one] (FTH-GlcVal).

46. (canceled)

47. (original) A container for use when analyzing adducts in a fluid or a solid material suspected of containing said adducts, wherein said container provides means for performing steps a) - c) as set out in claim 1.

48. (canceled)

49. (previously presented) A kit containing standard material according to claim 44.

50. (previously presented) A kit containing a compound according to claim 45 and a container.

51. (previously presented) An apparatus for performing the method according to claim 1 and providing means for performing steps a) - c) and for the detection in step d).

52. (previously presented) A computer program stored on a data carrier for performing the method according to claim 1.

53. (new) A method according to claim 10, wherein said size-discriminating ultrafiltration is followed by an ion-exchanging step.

54. (new) A method according to claim 10 wherein said ultracentrifugation is followed by an ion-exchanging step.

55. (new) A method according to claim 14 wherein the detection of the analyte in step d) is performed at a pH of approximately 7.

56. (new) A method according to claim 21 wherein said fluid and/or solid material is blood or processed blood of human origin.

57. (new) A method according to claim 21 wherein said fluid and/or solid material is blood or processed blood which has been obtained at an earlier stage contained in a container.

58. (new) A method according to claim 57 wherein said fluid and/or solid material is blood or processed blood which has been obtained at an earlier stage contained in a tube.

59. (new) A method according to claim 24 wherein said centrifugation, washing and lysating is followed by heating at approximately 70°C for approximately 1 hour.

60. (new) A method according to claim 25 wherein said lysating only, is followed by heating at approximately 38°C for approximately 18 hours.

61. (new) A method according to claim 28 wherein the purifying of said analyte is performed by first washing the resin

to which the analyte is bound and release the analyte from the resin by adding an acid to said resin, and subsequently filter the resin off giving the analyte in the remaining filtrate.